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Immunology

Blocking of pregnancy in mice by immunization with anti-idiotype directed against monoclonal anti-progesterone antibody

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ABSTRACT **Passive transfer of monoclonal anti-progesterone antibodies shortly after mating blocks the onset of pregnancy in different species (mouse, rat, and ferret). Here we report that BALB/c mice can be actively immunized against progesterone, and hence against pregnancy, by means of rabbit anti-idiotypic antibodies specific for a mouse monoclonal anti-progesterone antibody, DB3. Some of the anti-idiotypic antibodies reacted with the steroid-combining site on the DB3 molecule. In response to repeated anti-idiotypic immunization, mice produced serum anti-progesterone antibodies (up to 100 μg/ml) that resembled DB3 in idiotypy, affinity, and specificity for progesterone and other steroid ligands. Thus an anti-idiotype can mimic the antigenicity of a steroid hormone with a high degree of accuracy. Compared with immunization with a progesterone-bovine serum albumin conjugate, the anti-progesterone response to anti-idiotype was considerably lower and clonally restricted. When mated after completion of the immunization course, the fertility rate of anti-idiotype-immunized mice was reduced to 30% from a control level of 91%. The anti-fertility effect was correlated with the circulating anti-progesterone concentration in individual animals and persisted for 4 or 5 estrous cycles. Active immunization with progesterone-bovine serum albumin was a highly effective means of rendering mice infertile; it reduced the fertility rate to zero over 16 or 17 estrous cycles. Our results suggest that anti-idiotypes may form the basis of contraceptive vaccines.**

Immunological intervention in pregnancy is of interest as a possible approach to contraception and the control of reproduction. The targets for such intervention include the hormones associated with pregnancy and antigens on gametes. Thus, recent work has focused on a vaccine against human chorionic gonadotrophin (1) and immunization against sperm-specific proteins (2). Progesterone, the key steroid hormone required for the establishment and maintenance of pregnancy, is another possible target molecule. Active anti-progesterone immunization with a progesterone-protein complex blocks pregnancy in rats (3) and rabbits (4), and we have shown that passive immunization with anti-progesterone monoclonal antibodies (mAbs) shortly after mating prevents implantation and pregnancy in mice (5, 6), rats (7), and ferrets (8). The action of anti-progesterone antibody involves sequestration of the hormone in the circulation, preventing it from reaching progesterone receptors in uterine tissue (9); in addition, we have recently shown that antibody becomes localized specifically on the surface of progesterone-sensitive areas of the uterus (luminal and glandular epithelium) just before the time of implantation, indicating the possibility of a local blocking action affecting hormone uptake (10).

Anti-idiotypes (Id) are antibodies that recognize the antigen-combining regions of other antibodies and as such have been widely used as antigen mimics and experimental vaccines (11, 12). A possible route to fertility regulation would be active immunization with appropriate anti-idiotypic antibodies, which might mimic progesterone and thereby lead to antibody production against the hormone (13). In this laboratory, polyclonal rabbit anti-Id have been raised against a mouse anti-progesterone mAb designated DB3; binding of anti-Id to DB3 was partially inhibitable by free progesterone, indicating the presence of antibodies reactive with the steroid-binding site (14). Here we report that active anti-idiotypic immunization with anti-DB3-Id is indeed able to imitate the antigenic properties of the steroid and can stimulate production of anti-progesterone antibodies resembling DB3 in affinity, specificity, and idiotypy. Moreover, a course of anti-idiotypic immunization prevented establishment of pregnancy in about 70% of treated mice; pregnancy blocking was correlated with the level of anti-progesterone antibody induced in the circulation and was reversible.

MATERIALS AND METHODS

Antigens. Progesterone was conjugated to proteins [bovine serum albumin (BSA) and ovalbumin] at the 11α position on the steroid nucleus by a succinyl ester bridge as described (14, 15). The molar coupling ratios were 10:1 (progesterone:BSA) and 17:1 (progesterone:ovalbumin).

Anti-Progesterone mAbs. Production and purification of mAbs against progesterone and their characteristics of specificity and affinity have been described (5, 6, 14). The principal antibody used in this study was DB3 (IgG1, $K_a = 0.2 \times 10^9$ liter/mol); others referred to are 10/8, 10/16, and 11/32 (all IgG1) and 11/64 (IgM).

Anti-Progesterone Anti-Id. Polyclonal anti-Id were prepared by immunization of New Zealand White rabbits (Morton Rabbits, Stansted, Essex, U.K.) with DB3 (14) and purified by affinity chromatography on normal mouse immunoglobulin (mIg) and DB3-Sepharose 4B (Pharmacia) columns (14, 16).

Rat monoclonal anti-DB3-Id 11/7 was obtained from a fusion between spleen cells of a DA rat injected three times with the purified $F(ab')_2$ fragment of DB3 and the rat myeloma line Y3 (17). Out of five monoclonal anti-progesterones tested, 11/7 reacted with DB3 and, to a considerably lesser extent, with 11/64 (18).

Other Immunoglobulins. Mouse IgG1 myeloma protein MOPC21 (P3) was purified from ascites by precipitation with 18% (wt/vol) sodium sulfate and ion-exchange chromatography. Normal mIgs were precipitated from BALB/c serum at 18% sodium sulfate, dialyzed against phosphate-buffered saline (PBS), and used without further purification.

Radioimmunoassays. (*i*) *Anti-progesterone solid-phase RIA.* Solid-phase RIA of anti-progesterone antibodies was

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Abbreviations: mAb, monoclonal antibody; BSA, bovine serum albumin; Id, idiotype(s); mIg, mouse immunoglobulin; V, variable; L, light; H, heavy.

performed by binding to progesterone-ovalbumin-coated microtiter wells and detecting bound antibody by ^{125}I-labeled sheep anti-mIg (Amersham) as described (14). (*ii*) *Anti-Id solid-phase RIA.* Wells of microtiter plates were treated with purified DB3, P3 myeloma protein, or mIg (3.0 μg/ml) overnight and blocked. Anti-Id-containing rabbit sera or column fractions were titrated in the wells, and bound antibody was detected with ^{125}I-labeled donkey anti-rabbit Ig (Amersham) (14). (*iii*) *Progesterone-glucuronide blocking assay for site-directed anti-Id.* The wells of a microtiter plate were coated with anti-progesterone mAbs and blocked as above. Anti-DB3-Id was titrated in the wells and incubated for 1 hr. After washing, progesterone-11α-glucuronide-[^{125}I]iodotyramine (Amersham) in 10% normal sheep serum/PBS was added (100 μl per well; 20,000 cpm) and incubated for an additional hour. The plate was washed, and the radioactivity in individual wells was determined. The percent inhibition was calculated by comparison with control wells without anti-Id. (*iv*) *Id solid-phase competitive RIA.* A competitive inhibition assay was used to quantitate DB3-related Id in mouse serum after immunization. DB3 was radioiodinated with ^{125}I by using 0.2% lactoperoxidase (Sigma) according to the manufacturer's instructions. Microtiter wells were coated overnight with purified rabbit anti-DB3-Id or rat anti-DB3-idiotypic mAb 11/7 (3.0 μg/ml) and blocked. (Note that the rabbit anti-Id used in the assay was raised in a different animal from that used for anti-idiotypic immunization.) Sera of immunized mice were titrated in the wells using 10% normal sheep serum/PBS containing 1% normal rabbit Ig as diluent (to block anti-rabbit Ig activity) and incubated for 1 hr. After washing, ^{125}I-labeled DB3 (20,000 cpm in 100 μl) was added and incubated for an additional 1 hr. The plates were washed and dried, and the radioactivity was measured. Id levels were read from a DB3 standard curve. (*v*) *[^{3}H]Progesterone competition assay for antibody specificity.* Antibody samples (i.e., DB3 ascitic fluid or pooled plasma samples taken from mice immunized with progesterone-BSA or anti-DB3-Id) were diluted to bind 50% of offered ^{3}H-labeled progesterone in the absence of competing steroid. Competitive binding studies were carried out with 100 μl of antibody sample, 100 μl of increasing concentrations of unlabeled steroid (see Table 1), and 100 μl of [^{3}H]progesterone (20,000 dpm in phosphate buffer, 89 Ci/mmol; 1 Ci = 37 GBq; Amersham) (see refs. 5 and 19). Affinity for progesterone was determined by Scatchard analysis (6). (*vi*) *Plasma progesterone.* Total progesterone was extracted from 100-μl plasma samples with 5.0 ml of diethyl ether on a shaker for 10 min; after decanting the ether phase and evaporating to dryness, progesterone was determined by RIA (6).

Immunizations and Fertility Test. Virgin female BALB/c mice (8–10 weeks old, Institute-raised) were housed in a light (14 hr of light/10 hr of dark, light off at 20.00 hr)- and temperature (22°C)-controlled room and fed a mouse and rat diet (Labsure; Christopher Hill Group, Poole, Dorset, U.K.). They were inoculated subcutaneously on the back with 20 μg of rabbit anti-DB3-Id and boosted four times at 2-week intervals with an equal amount in incomplete Freund's adjuvant (once) or PBS (three times). Control animals received an equivalent regimen of either rabbit anti-mIg (affinity purified from the same hyperimmunized rabbit serum as the anti-Id) or the progesterone-BSA conjugate. Blood was taken from the tail vein at the end of each interval, and serum was assayed for anti-progesterone and DB3-related Id. Ten days after the last inoculation, immunized mice were caged with males of the same strain overnight, and vaginal plugs were checked the following morning (day 1 of pregnancy) (20). Where no copulation plug was found, animals were recaged with males on subsequent nights. Final blood samples were taken under ether anesthesia on day 10 after mating, and autopsies were performed to record the numbers of corpora lutea and implantation sites.

RESULTS

Specificity of Anti-Progesterone Anti-Id. Purified rabbit anti-DB3-Id showed specific binding to anti-progesterone mAb DB3 and virtually no reaction with normal mIg or P3 myeloma protein (Fig. 1*a*). When tested against other anti-progesterone mAbs from our panel, all of which are coded by closely related heavy (H)- and light (L)-chain variable (V) region genes (21, 22), significant cross-reactivity was found (Fig. 1*a*). Binding of ^{125}I-labeled progesterone-11α-glucuronide-[^{125}I]iodotyramine by DB3 and other mAbs was partially blocked by rabbit anti-DB3-Id (Fig. 1*b*).

Anti-Idiotypic Immunization Against Progesterone. Mice immunized with anti-DB3-Id responded with production of anti-progesterone antibody; the level increased progressively with repeated immunization to a peak of about 60 μg/ml after four inoculations. Control mice receiving rabbit anti-mIg did not produce anti-progesterone (Fig. 2). In the final blood sample taken at autopsy 3 weeks after the fifth inoculation of anti-Id, the mean level of anti-progesterone was 44.2 ± 7.1 μg/ml (see Table 2) and ranged from 5 to 97 μg/ml in individual animals (see Fig. 4). Immunization with progesterone-BSA induced very high levels of circulating anti-

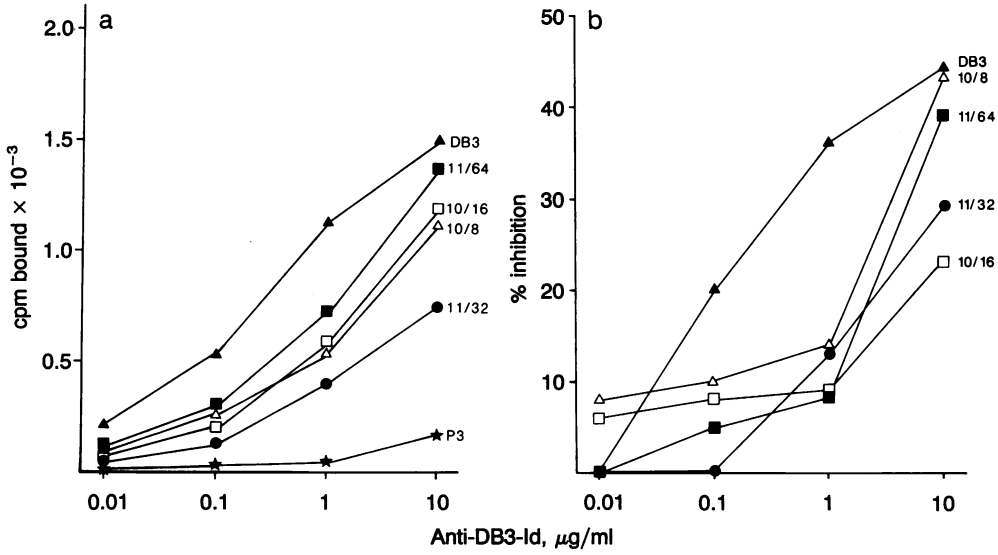

FIG. 1. Reaction between rabbit anti-DB3-Id and anti-progesterone mAbs. (*a*) Binding curves of purified rabbit anti-DB3-Id against the immobilized anti-progesterone mAbs DB3, 10/8, 10/16, 11/32, and 11/64 and the IgG1 myeloma protein P3, as determined by RIA. Binding to normal mIg was identical to that shown for P3. (*b*) Inhibition by rabbit anti-DB3-Id of binding of progesterone-11α-glucuronide-[^{125}I]iodotyramine to anti-progesterone mAbs. cpm bound in the absence of anti-Id were 889 (DB3), 461 (10/8), 508 (10/16), 377 (11/32), and 584 (11/64).

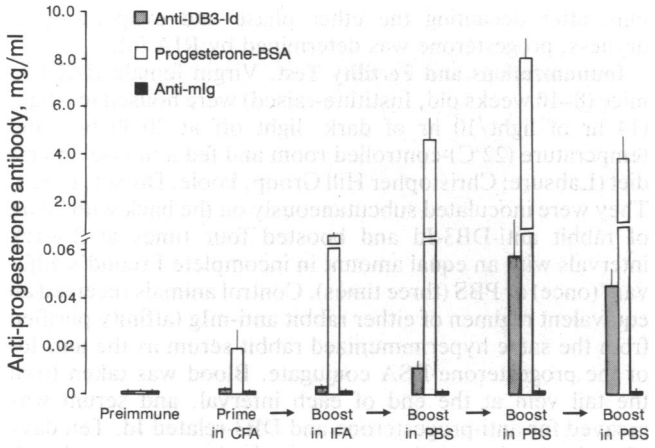

FIG. 2. Serum anti-progesterone responses of mice immunized with rabbit anti-DB3-Id (20 mice), progesterone-BSA conjugate (12 mice), or rabbit anti-mIg (11 mice). Immunizations were at 2-week intervals; serum was taken at the end of each interval and anti-progesterone levels were determined by RIA. CFA, complete Freund's adjuvant; IFA, incomplete Freund's adjuvant.

progesterone (mean of 3.8 ± 0.9 mg/ml after five inoculations).

The anti-progesterone mAbs show individual patterns of cross-reactivity with different steroids (fine-specificity), which are characteristic of each antibody (5, 6). The affinity for progesterone of the serum antibodies induced by anti-DB3-Id and their fine-specificity for five different steroids closely resembled those of DB3 itself and the polyclonal antisera raised by progesterone-BSA (Table 1). In the one instance where the specificity of DB3 diverges significantly from polyclonal anti-progesterone—namely, cross-reaction with etiocholanolone—the antibodies raised by anti-DB3-Id resembled DB3.

Quantitative determination of DB3-Id levels in serum showed that, at each time point, only a small fraction (0.5–1%) of the antibody response to progesterone-BSA consisted of DB3 Id (Fig. 3*a*), but, after anti-Id immunization, the level of this Id could account for virtually the entire anti-progesterone response (Fig. 3*b*). The circulating concentration of Id-bearing molecules never exceeded that of anti-progesterone antibody, and the difference between anti-progesterone and DB3-Id levels after anti-idiotypic immunization was generally not significant. We further determined the serum levels of a private DB3 Id defined by a rat mAb (11/7); the 11/7 idiotope was present at 6–8% of the anti-progesterone concentration after anti-Id immunization but

Table 1. Specificity and affinity of anti-progesterone mAb DB3 and BALB/c serum antibodies raised against anti-DB3-Id or progesterone-BSA

Steroid	DB3 ascites	Anti-DB3-Id immunized	Progesterone-BSA immunized
	% cross-reactivity		
Progesterone	100	100	100
11α-Hydroxyprogesterone	128	108	117
5α-Pregnane-3,20-dione	33	31	42
5β-Pregnan-3α-ol-20-one	3	3	7
Etiocholanolone	54	33	116
Pregnenolone	1	5	7
	K_a, liters/mol		
Progesterone	0.12×10^9	0.25×10^9	0.5×10^9

Binding to different steroids was determined by competition with ^{3}H-labeled progesterone, and affinity was determined by Scatchard analysis.

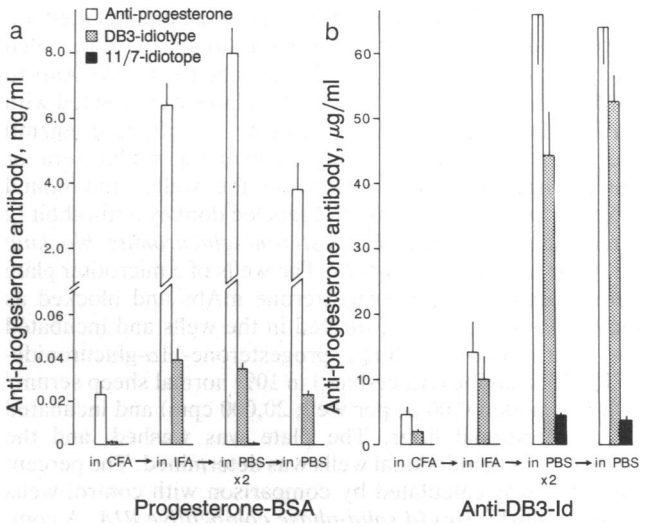

FIG. 3. Serum anti-progesterone, DB3-Id, and 11/7 idiotope levels in mice immunized with progesterone-BSA (*a*) or rabbit anti-DB3-Id (*b*). Protocol of immunizations and bleeds were as in Fig. 2 (results presented here are an independent experiment). Levels of anti-progesterone, DB3-Id, and 11/7 idiotope were determined by RIA. CFA, complete Freund's adjuvant; IFA, incomplete Freund's adjuvant.

was virtually undetectable on antibodies raised by progesterone-BSA (0.006%) (Fig. 3). This implies that, although most of the anti-progesterone antibodies raised by anti-Id are similar in combining specificity and Id to DB3, they are probably not identical to DB3.

Fertility of Anti-Id Immunized Mice. As shown in Table 2, the pregnancy rate of control mice immunized with rabbit anti-mIg was 90.9%, whereas in mice immunized with rabbit anti-DB3-Id the rate fell to 30%. The outcome in individual mice is shown in Fig. 4, from which it is seen that all mice that became pregnant in the anti-Id group had anti-progesterone levels of less than 15 μg/ml; those with anti-progesterone levels of 30 μg/ml or more were all infertile. Statistically, there was a clear positive correlation in anti-Id-treated animals between pregnancy blocking and the level of circulating anti-progesterone antibody induced: the 14 mated, nonpregnant mice had a significantly higher level of circulating antibody (59.8 ± 6.5 μg/ml) than the 6 pregnant mice (7.8 ± 0.8 μg/ml; $P < 0.001$). None of the mice immunized with progesterone-BSA became pregnant (Fig. 4).

Plasma Progesterone Levels and Corpora Lutea After Immunization. The circulating progesterone concentration at day 10 of pregnancy in untreated or anti-mIg-immunized mice was about 30 ng/ml; immunization with anti-Id led to an increase of about 100%, while immunization with progesterone-BSA increased the level by over 700% (Table 3). The rise in plasma progesterone thus correlates with the level of anti-progesterone antibody determined by RIA and also demonstrates that the antibody binds plasma progesterone *in vivo* (21, 22). Interestingly, active immunization with anti-Id or progesterone-BSA did not influence the ovulation rate, since the number of corpora lutea was normal and all the mice involved had vaginal plugs after being caged with males (Table 3).

Reversibility of Anti-Fertility Effect of Anti-Progesterone Immunization. To determine the duration of the anti-fertility effect, groups of 10 mice immunized by the above regimes were caged with males 10 days after the final inoculation, and the time between first detection of a copulation plug to delivery of pups was observed. In the control group treated with anti-mIg, pups were delivered at 21.9 ± 1.2 days (the normal gestation period), whereas in anti-Id-immunized an-

Table 2. Active immunization against pregnancy in BALB/c mice by anti-DB3-Id or progesterone-BSA

Immunization	Anti-progesterone level at day 10 after mating, μg/ml	No. pregnant/ No. mated	Implantation sites per mouse	Pregnancy rate, %
Anti-DB3-Id	44.2 ± 7.1	6/20	2.6 ± 0.9	30.0
	59.8 ± 6.5 (NP)			
	7.8 ± 0.8 (P)			
Progesterone-BSA	3800 ± 900	0/12	0	0
Anti-mIg	0.49 ± 0.04	10/11	8.5 ± 1.1	90.9

Female mice immunized five times with anti-DB3-Id, progesterone-BSA, or anti-mIg were mated, and pregnancy was determined at autopsy on day 10 after mating. Plasma anti-progesterone was determined by RIA on samples taken on the day of autopsy. Values given are the means ± SEM. NP, nonpregnant; P, pregnant animals in the group immunized with anti-DB3-Id.

imals this was prolonged to 29.1 ± 3.3 days. In progesterone-BSA-immunized mice, the interval was 78.7 ± 8.7 days. By taking into account the 10-day interval before mating, the duration of infertility after anti-Id immunization was about 20 days, or 4 or 5 estrous cycles, whereas after progesterone-BSA immunization mice were infertile for about 70 days or 16 or 17 cycles. In animals becoming pregnant for the first time after progesterone-BSA immunization, litter size was reduced significantly, from 8.2 ± 0.8 (control) to 5.0 ± 0.6 (37.5%, $P < 0.01$); the effect of anti-idiotypic immunization on litter size was marginal (6.3 ± 1.1). The body weight of the pups 21 days after delivery was normal in all groups (about 10 g).

DISCUSSION

The starting point of these experiments was the anti-progesterone mAb DB3, previously shown to have the effect of blocking implantation and pregnancy if injected into BALB/c mice 32–65 hr after mating (5). In this paper, we have demonstrated (*i*) that an anti-idiotypic antibody against DB3 can mimic the immunogenicity of the steroid hormone, making it possible to raise anti-progesterone antibodies without use of a steroid-protein conjugate; (*ii*) that anti-DB3-Id immunization gives rise to a restricted, "DB3-like" antibody response; and (*iii*) that the induced antibody has the predicted effect of blocking pregnancy. These observations suggest

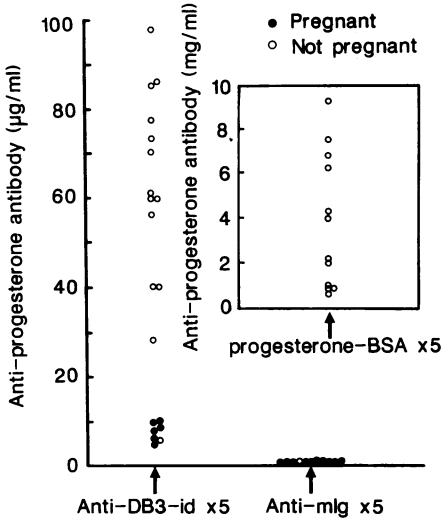

FIG. 4. Pregnancy and plasma anti-progesterone levels of individual BALB/c mice after immunization with anti-DB3-Id, anti-mIg, or progesterone-BSA. The treatment of mice in the three groups is indicated. Anti-progesterone levels were determined by RIA on plasma samples taken at autopsy on day 10 of pregnancy. Pregnancy status of individual animals is plotted against anti-progesterone concentration in plasma.

that anti-Id against reproductive hormones such as progesterone may form the basis of contraceptive vaccines and, in the wider context, that anti-Id may be a useful means of raising antibodies of defined specificity against autologous hormones and modifying their physiological effects.

Messenger RNA sequencing of several anti-progesterone mAbs from our panel, including DB3, has shown that V_H and V_L are derived from a restricted set of V genes (23, 24). The structural relatedness of anti-progesterone antibodies is reflected in the cross-reaction of the rabbit anti-DB3-Id with different members of the panel (Fig. 1*a*). By direct binding, cross-reactivity was highest against 11/64, a molecule that is 90% and 93% identical to DB3 in the V_H and V_L primary sequences, respectively, and less against 11/32, which is 83% identical to DB3 in V_H and 94% identical to DB3 in V_L. A significant proportion of the anti-DB3-idiotypic molecules appear to react with private idiotopes on DB3, probably partly generated by somatic mutation (25–27) during the development of the response (DB3 was derived after hyperimmunization). The private nature of the DB3 Id is also seen in the fact that only about 1% of serum anti-progesterone raised against progesterone-BSA is idiotypically DB3-like by competition assay (Fig. 3). Thus, the DB3 molecule can be considered one of many possible high-affinity end products of clonal maturation in the anti-progesterone response.

Some of the antibodies in the rabbit anti-DB3-Id recognize antigen combining-site-related idiotopes, as indicated by partial blocking of progesterone-glucuronide binding; the combining-site-related idiotopes are among those that are shared between different anti-progesterones (Fig. 1*b*). Reactivity with the steroid-binding site itself is probably essential for antigenic mimicry of the steroid by anti-Id, the so-called "internal image" effect of anti-Id (28). The steroid reactivity of serum anti-progesterone raised by anti-DB3-Id was strikingly similar in its detail to that of DB3 (Table 1), both in affinity for progesterone and reactivity with five other steroids, demonstrating that anti-idiotypic mimicry of progesterone is highly accurate.

Table 3. Plasma progesterone levels and corpora lutea at autopsy 10 days after mating in mice immunized with anti-DB3-Id, anti-mIg, or progesterone-BSA

Immunization	No. of mice	Corpora lutea per mouse	Plasma progesterone, ng/ml
None	22	11.3 ± 0.4	28.9 ± 2.4
Anti-DB3-Id	20	10.8 ± 0.6	60.0 ± 6.4
Progesterone-BSA	12	13.0 ± 0.5	257.7 ± 43.9
Anti-mIg	11	10.6 ± 0.5	30.5 ± 3.4

Total plasma progesterone levels were assayed by ether extraction and RIA, and the number of corpora lutea were counted at autopsy (day 10 of pregnancy). Values given are the means ± SEM.

The level of anti-progesterone induced by anti-DB3-Id is about 1% of that induced by progesterone-BSA and is close to the level of DB3-bearing molecules present in the response to that conjugate (Figs. 2 and 3). The selective effect of anti-Id is clearly seen in the concordance between the levels of anti-progesterone and DB3-related Id in the serum of anti-Id-immunized mice; virtually all the anti-progesterone antibodies raised by anti-Id can thus be accounted for as DB3-like, whereas this would only be true of some 1% of the antibodies raised by progesterone-BSA. It is also noteworthy that there is no excess of idiotypic molecules over anti-progesterone at any stage (Fig. 3*b*). On the other hand, the finding that only 6–8% of anti-progesterones carry the private DB3 idiotope 11/7 (defined by a rat anti-DB3 mAb) indicates that the antibody population induced by anti-Id is heterogeneous and that no more than 6–8% can be identical to DB3.

Anti-idiotypic immunization was an effective means of providing a period of temporary infertility. Pregnancy blocking showed a clear threshold, in that only mice making over 15 μg of circulating anti-progesterone antibody per ml became infertile. With anti-DB3-Id used in our regime, 6 out of 20 mice immunized with anti-Id failed to reach this threshold and became pregnant when mated (Fig. 4). From a separate group of similarly treated mice, it appeared that protection from pregnancy lasted until the antibody level fell below this threshold (data not shown). The mean anti-progesterone level induced by anti-Id in all mice was 44 μg/ml, and the pregnancy rate was 30%. This agrees closely with the efficacy of passively transferred DB3, where, to achieve a 50% pregnancy-blocking rate in BALB/c mice, about 120 μg of DB3 antibody as a single intraperitoneal injection was required, with a circulating level 6 hr later of about 50 μg/ml (6).

Based on our studies of passive immunization, the mechanism of pregnancy blocking by anti-idiotypic immunization is likely to be a failure of implantation of the fertilized embryo, brought about by the fall in available plasma progesterone combined with local effects of anti-progesterone antibody in the uterus (9, 10, 21, 22). To support this, we have shown here that the total plasma progesterone rises after immunization, in agreement with binding of the hormone by circulating antibody and the consequent prolongation of its half-life (Table 3). Furthermore, ovulation is apparently unaffected by progesterone immunization, since the number of corpora lutea in immunized, nonpregnant mice was normal (Table 3); effects on tubular transport of ova or embryos and on embryonic development cannot be ruled out (21, 22). The possibility should also be considered that anti-Id might exert an anti-fertility effect through receptor blockade, since in some cases anti-idiotypic mimicry is manifested as a cross-reaction with cellular receptors (29, 30). However, this seems unlikely here, first because progesterone receptors are intracellular (31) and second because of the close correlation between induced plasma anti-progesterone level and the anti-fertility effect (Fig. 4).

Infertility resulting from anti-Id or progesterone-BSA immunization is reversed with time, partly because continuous progesterone production will eventually block most of the antibody sites. As expected from the respective anti-progesterone levels induced, immunization with the steroid conjugate is considerably more potent, producing complete pregnancy block in all animals for over 2 months (mean of 16 or 17 estrous cycles). Immunization with anti-DB3-Id is less effective in both respects and is more readily reversed (after 4 or 5 estrous cycles). As far as design of a contraceptive vaccine is concerned, efficacy is obviously crucial, but so too is the possibility of reversal, and in this respect anti-idiotypic immunization appears to offer some advantages.

In conclusion, we have reported here an application of anti-idiotypic immunization not only in inducing the immune response against a steroid hormone but also in affecting as a consequence a normal physiological process, fertility. Our results demonstrate that it is possible to regulate the activity of a hormone *in vivo* through the induction of autoantibodies (anti-progesterone) by means of anti-idiotypic immunization. They thus provide a model for immunological control of hormone-dependent processes in general by using anti-Id to replace the hormone in the immunization procedure.

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